



This is a repository copy of *Disrupted glycosylation of lipids and proteins is a cause of neurodegeneration*.

White Rose Research Online URL for this paper:
<http://eprints.whiterose.ac.uk/161801/>

Version: Published Version

Article:

Moll, T., Shaw, P.J. orcid.org/0000-0002-8925-2567 and Cooper-Knock, J. orcid.org/0000-0002-0873-8689 (2020) Disrupted glycosylation of lipids and proteins is a cause of neurodegeneration. *Brain*, 143 (5). pp. 1332-1340. ISSN 0006-8950

<https://doi.org/10.1093/brain/awz358>

Reuse

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial (CC BY-NC) licence. This licence allows you to remix, tweak, and build upon this work non-commercially, and any new works must also acknowledge the authors and be non-commercial. You don't have to license any derivative works on the same terms. More information and the full terms of the licence here:
<https://creativecommons.org/licenses/>

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk
<https://eprints.whiterose.ac.uk/>

UPDATE**Disrupted glycosylation of lipids and proteins is a cause of neurodegeneration****Tobias Moll, Pamela J. Shaw and  Johnathan Cooper-Knock**

Glycosyltransferases represent a large family of enzymes that catalyse the biosynthesis of oligosaccharides, polysaccharides, and glycoconjugates. A number of studies have implicated glycosyltransferases in the pathogenesis of neurodegenerative diseases but differentiating cause from effect has been difficult. We have recently discovered that mutations proximal to the substrate binding site of glycosyltransferase 8 domain containing 1 (GLT8D1) are associated with familial amyotrophic lateral sclerosis (ALS). We demonstrated that ALS-associated mutations reduce activity of the enzyme suggesting a loss-of-function mechanism that is an attractive therapeutic target. Our work is the first evidence that isolated dysfunction of a glycosyltransferase is sufficient to cause a neurodegenerative disease, but connection between neurodegeneration and genetic variation within glycosyltransferases is not new. Previous studies have identified associations between mutations in *UGT8* and sporadic ALS, and between *ST6GAL1* mutations and conversion of mild cognitive impairment into clinical Alzheimer's disease. In this review we consider potential mechanisms connecting glycosyltransferase dysfunction to neurodegeneration. The most prominent candidates are ganglioside synthesis and impaired addition of *O*-linked β -*N*-acetylglucosamine (*O*-GlcNAc) groups to proteins important for axonal and synaptic function. Special consideration is given to examples where genetic mutations within glycosyltransferases are associated with neurodegeneration in recognition of the fact that these changes are likely to be upstream causes present from birth.

Sheffield Institute for Translational Neuroscience (SITraN), Sheffield, UK

Correspondence to: Dr Johnathan Cooper-Knock
Sheffield Institute for Translational Neuroscience (SITraN)
University of Sheffield
385 Glossop Road, Sheffield S10 2HQ, UK
E-mail: j.cooper-knock@sheffield.ac.uk

Keywords: glycosyltransferase; neurodegeneration; gangliosides; *O*-linked β -*N*-acetylglucosamine**Abbreviations:** ALS = amyotrophic lateral sclerosis; *O*-GlcNAc = *O*-linked β -*N*-acetylglucosamine**Introduction**

Glycosyltransferases represent a large family of enzymes that catalyse biosynthesis of oligosaccharides, polysaccharides, and glycoconjugates. Sugar moieties are transferred from activated sugar donors to specific acceptor molecules via the formation of glycosidic bonds (Chuh *et al.*, 2016). Acceptor molecules include other sugars, nucleic acids, lipids, and proteins. Glycosyltransferases reside predominantly within the Golgi apparatus of eukaryotes as type II transmembrane proteins. Over 90 glycosyltransferase

families have been described (www.cazy.org/GlycosylTransferases.html). Sequence alignment tools have been useful for predicting glycosyltransferase function, including a metal-binding motif important for configuration of substrate within the active site (Lairson *et al.*, 2008). However, even closely related sequences have been shown to exhibit different catalytic activity (Breton *et al.*, 2006). Glycosyltransferases are classified as either 'retaining' or 'inverting' enzymes according to whether the anomeric bond within the donor substrate is retained or inverted during the sugar transfer.

Received July 9, 2019. Revised September 10, 2019. Accepted September 16, 2019. Advance Access publication November 14, 2019

© The Author(s) (2019). Published by Oxford University Press on behalf of the Guarantors of Brain.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Neurodegenerative diseases are increasing in frequency, in part due to an ageing population. Despite this, neurodegenerative diseases represent a significant unmet health need without effective treatments or clearly delineated pathogenic mechanisms. Changes in expression levels of glycosyltransferases have been strongly linked with neurodegeneration (Ludemann *et al.*, 2005; Desplats *et al.*, 2007; Schneider 2018), but determining whether these effects are upstream of neurotoxicity is difficult. Two distinct glycosyltransferase-associated mechanisms are prominent: ganglioside synthesis and addition of *O*-linked β -*N*-acetylglucosamine to proteins (*O*-GlcNAcylation). Major gangliosides are sialic acid-containing glycosphingolipids. Within the mammalian brain they are synthesized in the endoplasmic reticulum from a lactosylceramide precursor before remodelling during transit from the *cis*- to the *trans*-Golgi network by a series of glycosyltransferase enzymes (Fig. 1). Mature gangliosides are expressed on the plasma membrane of most vertebrate cells and within bodily fluids. They are particularly abundant on neuronal

and glial cells within the CNS where they are thought to function prominently in cell signalling (Vajn *et al.*, 2013). Altered levels of gangliosides have been reported in animal models of amyotrophic lateral sclerosis (ALS) and in post-mortem CNS tissue from ALS patients (Ariga, 2014; Dodge *et al.*, 2015); similar findings have been reported in Parkinson's disease (Wu *et al.*, 2012) and Alzheimer's disease (Gyls *et al.*, 2007). *O*-GlcNAcylation occurs predominantly in the brain and is regulated by the glycosyltransferases *O*-linked *N*-acetylglucosamine transferase (OGT) and EGF domain-specific *O*-linked *N*-acetylglucosamine transferase (EOGT), which attach the *O*-GlcNAc moiety to acceptor proteins at specific serine/threonine residues via an *O*-linked glycosidic bond. OGT acts intracellularly whereas EOGT acts extracellularly on secreted and membrane proteins (Fig. 2). *O*-GlcNAcylation of CNS proteins important for axonal and synaptic function is significantly reduced in animal models of neurodegenerative diseases and in patient tissue from diseases including Parkinson's disease, Huntington's

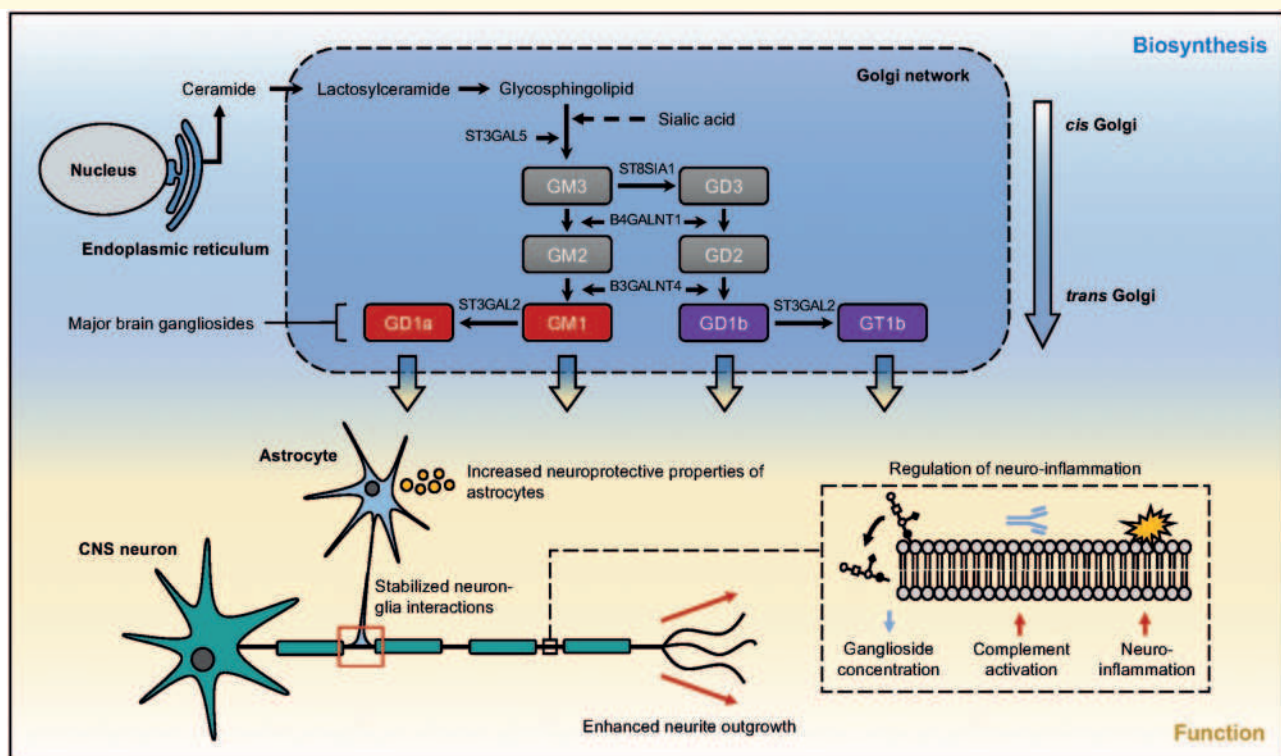


Figure 1 Schematic overview of the biosynthesis and function of major gangliosides within the mammalian brain.

Lactosylceramide is synthesized at the cytoplasmic leaflet of the endoplasmic reticulum membrane from its ceramide precursor. *De novo* ceramide is transported to the Golgi apparatus and is converted to glycosphingolipids and sphingomyelin through the addition of saccharides and phosphocholine, respectively. Glycosphingolipids are transported in vesicles to the outer leaflet of the plasma membrane. Sialic acid-enriched glycosphingolipids form gangliosides which are anchored to the membrane via their ceramide-lipid moiety. Four major gangliosides comprise > 90% of total gangliosides within the brain. A-series gangliosides (red) are derived from GM3. B-series gangliosides (purple) are synthesized from GM3 by GD3 synthase (St8sia1). G = the 'ganglioside' core; the second letter designates the quantity of sialic acid residues; M = mono; D = di; T = tri. Gangliosides are essential to maintaining neuronal integrity with functions including, but not limited to, increasing the neuroprotective properties of astrocytes, stabilizing interactions between neurons and glia, enhancing neurite outgrowth and negatively regulating neuroinflammation through activation of the complement pathway.

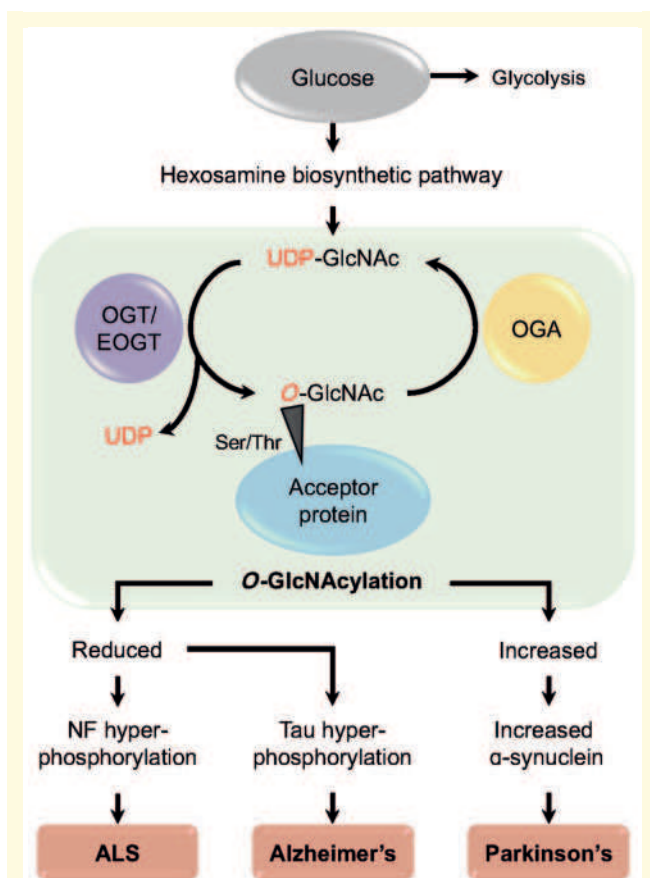


Figure 2 O-GlcNAcylation is implicated the pathophysiology of neurodegenerative disease. An overview of O-GlcNAcylation, a post-translational modification of O-GlcNAc, which has been implicated in neurodegenerative diseases Huntington's disease, Alzheimer's disease, Parkinson's disease and ALS. O-GlcNAcylation occurs predominantly in the brain and is regulated by the glycosyltransferases OGT and EOGT, which attach the O-GlcNAc moiety to acceptor proteins at specific serine/threonine residues via an O-linked glycosidic bond; OGT acts intracellularly whereas EOGT acts extracellularly on secreted and membrane proteins.

disease, Alzheimer's disease and ALS (Liu *et al.*, 2004; Ludemann *et al.*, 2005; Kumar *et al.*, 2014; Frenkel-Pinter *et al.*, 2017).

Neurodegenerative diseases exhibit late age of onset and it is therefore assumed that genetic mutations are upstream of disease pathogenesis. As a result, the discovery of neurodegenerative disease-associated DNA mutations is a significant step towards identification of upstream therapeutic targets. We have recently discovered that mutations proximal to the substrate-binding site of glycosyltransferase 8 domain-containing 1 (GLT8D1) disrupt enzyme activity and are associated with familial ALS (Cooper-Knock *et al.*, 2019). Our work is the first evidence that dysfunction of a glycosyltransferase is sufficient to cause a neurodegenerative disease. Our data are consistent with an effect of GLT8D1 mutations on ganglioside synthesis. In support of

this mechanism, we have demonstrated by immunocytochemistry that ALS-associated GLT8D1 mutations reduce membrane expression of glycosphingolipids, which include gangliosides, in human cells (unpublished data). Moreover, in this review we summarize previous literature linking genetic changes within glycosyltransferases to neurodegeneration, and provide new evidence that genetic mutations within EOGT are significantly associated with sporadic ALS making this another upstream therapeutic target.

Impaired ganglioside synthesis is linked to neurodegeneration

Parkinson's disease

Reduced glycosyltransferase expression and lowered ganglioside synthesis has been implicated in the pathogenesis of Parkinson's disease. A recent report described a reduction in gene expression of the glycosyltransferases *B3GALT4* and *ST3GAL2* in neuromelanin-containing neurons in the substantia nigra of patients with Parkinson's disease compared to control subjects (Schneider, 2018). These genes are key players in the ganglioside biosynthesis pathway (Fig. 1). It is proposed that reduced *B3GALT4* and *ST3GAL2* expression leads to vulnerability of dopaminergic neurons via aberrant ganglioside synthesis. Consistent with this hypothesis, the number of GM1 ganglioside-expressing cells in the Parkinson's disease substantia nigra are reduced (Wu *et al.*, 2012), and levels of the major brain gangliosides—GM1, GD1a, GD1b and GT1b—are decreased in whole substantia nigra homogenates from patients with Parkinson's disease (Seyfried *et al.*, 2018). Model systems provide evidence that dysfunction of ganglioside synthesis is a cause and not just an association of typical Parkinson's disease pathology: genetically engineered mice lacking major brain gangliosides display overt motor impairment with increasing age, which is accompanied by loss of dopaminergic neurons from the substantia nigra pars compacta and aggregation of α -synuclein (Wu *et al.*, 2012).

Huntington's disease

In a similar manner to Parkinson's disease, reduced expression of glycosyltransferases involved in ganglioside synthesis has also been described in the R6/1 mouse model of Huntington's disease and in human Huntington's disease patients (Desplats *et al.*, 2007). In this study >80% of gene expression changes observed in the striatum of R6/1 mice were also observed in the post-mortem caudate of human Huntington's disease subjects. Overlapping genes were significantly enriched with glycosyltransferases involved in ganglioside synthesis including *ST3GAL5*, *ST8SIA3*, *B4GALNT1* and *ST3GAL2* (Fig. 1). Consistent with impaired ganglioside synthesis, the same study

reported reduced ganglioside concentrations within both the diseased human caudate and the mouse striatum. It should be noted that despite significant homology to *ST8SIA1*, which has a well described role in ganglioside biosynthesis (Fig. 1), *ST8SIA3* is traditionally associated with *N*-glycosylation of secreted/membrane proteins within the CNS (Lin *et al.*, 2019). Like gangliosides, *N*-glycosylated proteins are important for cell signalling.

Alzheimer's disease

There is good evidence for perturbed ganglioside metabolism in patients with Alzheimer's disease, and in the development of amyloid- β pathology in particular (Barrier *et al.*, 2007). In contrast to the findings in Parkinson's disease and Huntington's disease, the key observation appears to be increased ganglioside synthesis. Elevated GM1, GM2 and GM3 levels have been reported in the cerebral cortices of Alzheimer's disease patients (Kracun *et al.*, 1992; Gyls *et al.*, 2007). Development of amyloid- β deposition is the defining pathology of Alzheimer's disease and within brains exhibiting early Alzheimer's disease pathology, a significant proportion of amyloid- β is bound to ganglioside species (Yanagisawa and Ihara, 1998). It has even been suggested that insoluble GM1-bound amyloid- β is the key toxin leading to neuronal death (Hayashi *et al.*, 2004), as a result of high affinity binding between GM1 and amyloid- β , which facilitates formation of insoluble β -pleated sheets (Yamamoto *et al.*, 2007). With increasing age GM1 is localized to presynaptic nerve terminals and this may have a role in directing amyloid- β deposition to the same locations (Yamamoto *et al.*, 2008). Unlike evidence regarding gangliosides, reports of altered glycosyltransferase expression in Alzheimer's disease are more limited. There is evidence that glycosyltransferase activity may modify Alzheimer's disease pathology: overexpression of the glycosyltransferase B4GALNT1 leads to increased ganglioside expression but also increases APP cleavage to form amyloid- β pathology through suppression of lysosomal degradation of BACE1 (Yamaguchi *et al.*, 2016). Currently, transgenic mouse models of Alzheimer's disease do not mirror changes in ganglioside distribution seen in human post-mortem tissue (Barrier *et al.*, 2007).

Amyotrophic lateral sclerosis

ALS has been linked to abnormal lipid metabolism (Desport *et al.*, 2005) and in particular, gangliosides and their ceramide precursors are thought to be modulators of disease progression (Salazargrueso *et al.*, 1990; Stevens *et al.*, 1993). Whether ganglioside production is increased or decreased is controversial. As early as 1985 a 10% reduction in b-series gangliosides was identified within the motor cortex of ALS brains compared to non-ALS controls (Rapport *et al.*, 1985). More recently elevated levels of gangliosides GM1 and GM3 were reported within ALS post-mortem spinal cords compared to age-matched

controls; findings were corroborated in the SOD1-G93A transgenic ALS mouse model (Dodge *et al.*, 2015). Interestingly, autoantibodies against specific gangliosides produce an inflammatory disease of spinal motor neurons known as multifocal motor neuropathy with conduction block (Harschnitz *et al.*, 2014), which is a frequent differential diagnosis of ALS.

ALS specifically inflicts pathology on the upper and lower motor neurons, the neuromuscular junction and muscle. The accessibility of this system in disease models facilitates the differentiation of up- and downstream disease associations. For example, increased expression of glycosphingolipids is observed in muscle tissue from end-stage mutant SOD1-ALS mice compared to controls, but similar changes were observed in response to surgically-induced muscle denervation suggesting a downstream effect (Henriques *et al.*, 2015). Moreover, neurotransmission at the neuromuscular junction is unchanged in aged GM2 and GD3-deficient mice compared to controls (Zitman *et al.*, 2011). However, our discovery that mutations in the glycosyltransferase *GLT8D1* are a cause of familial ALS is a step forward, which places glycosyltransferase activity irrefutably upstream in the development of disease.

Genetic mutations in glycosyltransferases cause neurodegeneration

Genetic mutations in the development of an age-associated neurodegenerative disease are, by definition, upstream causes or risk factors rather than secondary to the disease process. Mutations discovered to date are included in Table 1 and described below.

GLT8D1

A recent study from our lab demonstrated that mutations within the glycosyltransferase domain of *GLT8D1* are associated with familial ALS (Cooper-Knock *et al.*, 2019). The function of *GLT8D1* is unknown, but it is ubiquitously expressed and localized to the Golgi apparatus. Based on sequence homology, *GLT8D1* is a member of glycosyltransferase family 8 and is expected to catalyse the transfer of a glycosyl group from a donor to an acceptor via a 'retaining' mechanism. Mutated *GLT8D1* carrying ALS-associated amino acid changes is toxic to neuronal and non-neuronal cell lines, and induces motor deficits in zebrafish embryos; these observations are consistent with a role in motor neuron degeneration. Interestingly, relative toxicity of ALS-associated mutations in model systems mirrors the clinical severity. Glycosyltransferase enzyme activity is reduced in the mutated form of *GLT8D1* commensurate with an increase in substrate affinity, which is predicted to impair cycling of substrate through the enzyme and thus reduce overall velocity (Cooper-Knock *et al.*, 2019).

Table 1 Defects affecting specific glycosyltransferase enzymes observed in neurodegenerative disease

Glycosyltransferase	Functional consequence	Neurodegenerative disorder	Defect observed	Reference
ST6GAL1	Disrupted cell surface signalling	Alzheimer's disease	DNA mutations	Lee <i>et al.</i> , 2017
B3GALT4	Reduced ganglioside biosynthesis (GD1b)	Parkinson's disease	Reduced gene expression	Schneider, 2018
ST3GAL2	Reduced ganglioside biosynthesis (GT1b)	Parkinson's disease Huntington's disease	Reduced gene expression	Schneider, 2018 Desplats <i>et al.</i> , 2007
B4GALNT1	Reduced ganglioside biosynthesis	Huntington's disease	Reduced gene expression	Desplats <i>et al.</i> , 2007
ST8SIA3	Implicated in ganglioside biosynthesis but described role in N-glycosylation	Huntington's disease	Reduced gene expression	Desplats <i>et al.</i> , 2007
ST3GAL5	Reduced ganglioside biosynthesis	Huntington's disease	Reduced gene expression	Desplats <i>et al.</i> , 2007
GLT8D1	Reduced membrane expression of glycosphingolipids	ALS	DNA mutations	Cooper-Knock <i>et al.</i> , 2019
UGT8	Disruption of myelin synthesis	ALS	DNA mutations	Pamphlett <i>et al.</i> , 2011
EOGT	Disruption of O-GlcNAcylation	ALS	DNA mutations	This article
OGT	Impaired O-GlcNAcylation	Alzheimer's disease ALS	Reduced concentration of O-GlcNAcylated proteins	Liu <i>et al.</i> , 2004 Ludemann <i>et al.</i> , 2005
OGT	Excessive O-GlcNAcylation	Parkinson's disease	Increased concentration of O-GlcNAcylated proteins	Wani <i>et al.</i> , 2017

Taken together, these data are consistent with loss-of-function toxicity. Our study is the first time inherited mutations that diminish glycosyltransferase enzyme activity have been associated with ALS. We have recently demonstrated by immunocytochemistry that ALS-associated mutations reduce membrane expression of glycosphingolipids in human cells (unpublished data). Glycosphingolipids include gangliosides and this would be consistent with disruption of ganglioside signalling within the CNS. GLT8D1 was recently identified as a risk gene for schizophrenia (Yang *et al.*, 2018), and while schizophrenia is not a neurodegenerative disorder, it is noteworthy that ALS and schizophrenia share common genetic risk (McLaughlin *et al.*, 2017).

UDP glycosyltransferase 8 (UGT8)

Like GLT8D1, UGT8 is a member of glycosyltransferase family 8. UGT8 functions in the biosynthesis of galactocerebroside, a sphingolipid that forms the myelin membrane in the central and peripheral nervous systems. Rare and potentially pathogenic copy number variants have been identified in the promoter region of *UGT8* following in an unbiased genome-wide screen for *de novo* DNA mutations in 12 trios including sporadic ALS patients and unaffected parents (Pamphlett *et al.*, 2011). Abnormal lipid biosynthesis and metabolism is a pathological hallmark of ALS (Dupuis *et al.*, 2008; Dorst *et al.*, 2011), therefore it is possible that UGT8 plays a role in the hypolipidaemia observed in ALS patients and the SOD1-G93A ALS mouse model (Kim *et al.*, 2011; Yang *et al.*, 2013). Mice lacking *Ugt8a*, the orthologue of *UGT8*, exhibit impaired locomotor activity and disruption in nerve conduction followed by degeneration of the myelin sheath (Bosio *et al.*, 1996; Coetzee *et al.*, 1996), which is rescued following transgenic expression of *UGT8A* (Zoller *et al.*, 2005).

Interestingly the rescue occurred with expression of *UGT8A* under a promoter exclusively expressed within oligodendrocytes, which is consistent with other evidence implicating these cells in ALS-associated neurodegeneration (Morrison *et al.*, 2013).

ST6 β -galactoside α -2,6-sialyltransferase I (ST6GAL1)

ST6GAL1 is an 'inverting' enzyme and a member of glycosyltransferase family 29. ST6GAL1 catalyses the transfer of sialic acid onto galactose-containing substrates including cell-surface signalling lipids and proteins (Garnham *et al.*, 2019). A genome-wide association study implicated polymorphisms within ST6GAL1 in the conversion of mild cognitive impairment into clinical Alzheimer's disease (Lee *et al.*, 2017). Interestingly ST6GAL1 is cleaved and occurs in a soluble form; this cleavage is mediated by BACE1 (Kitazume *et al.*, 2001), which is also involved in the cleavage of APP to form amyloid- β . Indeed, overexpression of ST6GAL1 increases APP secretion (Nakagawa *et al.*, 2006) suggesting that the activity of ST6GAL1 can directly modify the central pathway in the development of Alzheimer's pathology.

Glycosyltransferase O-GlcNAcylation: a key regulator of neurodegeneration?

Protein glycosylation and more specifically the addition of O-GlcNAc groups to CNS proteins important for axonal and synaptic function, is significantly reduced in animal models of neurodegenerative diseases and in patient tissue from diseases

including Huntington's disease, Alzheimer's disease and ALS (Liu *et al.*, 2004; Ludemann *et al.*, 2005; Kumar *et al.*, 2014; Frenkel-Pinter *et al.*, 2017) (Table 1). O-GlcNAcylation is reported to negatively regulate tau phosphorylation (Liu *et al.*, 2004), which is key in the pathogenesis of a number of neurodegenerative diseases, including Alzheimer's disease. In contrast, an increase in O-GlcNAcylation is observed in the post-mortem temporal cortex of patients with Parkinson's disease and is postulated to contribute to neurodegeneration through the inhibition of autophagy leading to an increase in α -synuclein accumulation (Wani *et al.*, 2017). Neurofilaments are critical components of the neuronal cytoskeleton that can undergo O-GlcNAcylation (Yuan *et al.*, 2012). Neurofilament levels are significantly higher in the serum and CSF of ALS patients compared to control subjects (Benatar *et al.*, 2018). This increase is thought to be a consequence of axonal damage. However, there is evidence that neurofilament damage may be upstream in the pathogenesis of ALS including the observation that increased phosphorylation of neurofilaments is associated with neurotoxicity (Julien, 1997). It is thought that phosphorylation and O-GlcNAcylation are reciprocal, meaning that reduced O-GlcNAcylation could precipitate harmful phosphorylation; indeed this has been observed in a transgenic rat model of SOD1-ALS (Ludemann *et al.*, 2005).

O-GlcNAcylation occurs predominantly in the brain and is regulated by the glycosyltransferases OGT and EOGG; the reverse reaction is catalysed by O-GlcNAcase (OGA). Together these reactions constitute a dynamic and reversible process (Fig. 2). OGT is an inverting enzyme and a member of glycosyltransferase family 41; OGT is highly enriched in the brain, where it is 10 times more active than in peripheral tissue (Okuyama and Marshall, 2003). OGT is localized to the nucleus, soma, dendrites and presynaptic terminals of neurons (Akimoto *et al.*, 2003). Removal of postsynaptic OGT from primary neurons inhibits both synapse formation and the development of dendritic spines (Lagerlof *et al.*, 2017). This highlights the importance of OGT in maintaining synaptic stability, and notably loss of synaptic stability is a unifying feature of neurodegenerative disease. EOGG is an inverting enzyme and a member of glycosyltransferase family 61. Despite distinct sites of action, OGT and EOGG are both regulated via the hexosamine biosynthetic pathway (Ogawa *et al.*, 2015). EOGG activity is involved in Notch signalling, which is important for neurodevelopment. Indeed, homozygous loss-of-function mutations in EOGG produce Adams-Oliver syndrome, a congenital developmental disorder associated with actin cytoskeleton defects.

ALS-associated genetic variants within O-GlcNAcylation pathway enzymes

While homozygous EOGG mutations affect neurodevelopment, we hypothesized that heterozygous mutations within

EOGG might negatively impact on the maintenance of axon integrity and increase risk of developing ALS. To test this hypothesis we performed rare-variant burden testing (Cirulli *et al.*, 2015) within EOGG to check for a genetic association with ALS. We used whole genome sequencing data from 4493 sporadic ALS patients and 1924 control subjects (van der Spek *et al.*, 2019); we identified 32 missense rare (MAF < 1%) variants within EOGG that were exclusively or predominantly found in ALS cases (Table 2). When considering all rare missense variants found in cases and controls across all exons of EOGG, there was a significant enrichment of such mutations in ALS patients (Firth logistic regression, $P = 0.007$). Similar testing did not identify an enrichment of ALS-associated mutations within OGT, indeed we only identified two rare missense mutations within OGT in 4493 sporadic ALS patients. It should be noted that OGT is encoded on the X chromosome and therefore males are necessarily hemizygous, which may predispose to a neurodevelopmental phenotype rather than a late age-of-onset disease: for example mutations within N-terminal tetratricopeptide repeats of OGT are associated with X-linked intellectual disability (Gundogdu *et al.*, 2018). There was no significant burden of ALS-associated mutations within OGA ($P = 0.91$).

Conclusions

Overall there is substantial evidence for dysfunction of glycosyltransferases in neurodegenerative diseases including ALS, Alzheimer's disease, Huntington's disease and Parkinson's disease. There are diverse functions associated with glycosyltransferase activity and for many of the enzymes the biological pathway associated with their activity is not yet clear. However, in our analysis, dysfunction associated with neurodegenerative disease can be seen to converge on the ganglioside synthesis pathway and altered O-GlcNAcylation. The exact nature of the defect appears to be variable in different diseases; for example ganglioside concentrations are reduced in Parkinson's disease and Huntington's disease, increased in Alzheimer's disease and there is evidence for change in both directions in ALS. Similarly, increased O-GlcNAcylation is associated with the development of Parkinson's disease pathology but reduced O-GlcNAcylation is associated with the development of tau pathology. We suggest that consensus will arise via efforts to position glycosyltransferase dysfunction within the cascade of pathogenesis leading to neuronal death—it is not glycosyltransferase dysfunction *per se* that is interesting, but rather upstream changes in glycosyltransferase function that initiate toxicity. With this in mind we have highlighted genetic associations between mutations in glycosyltransferases and neurodegenerative disease. Most prominently we have recently discovered autosomal dominant mutations in GLT8D1 to be a monogenic cause of ALS. Disease-associated mutations have also been discovered in UGT8 and ST6GAL1; and we have revealed a

Table 2 Mutations in EOGT found in ALS patients

DNA change	Protein change	Allele frequency		Exon
		ALS	Controls	
c.1575T>G	p.Asp525Glu	0.001	0.0005	15
c.1546C>T	p.Pro516Ser	0.0001	0	15
c.1466C>T	p.Pro489Leu	0.0001	0	15
c.1459dupG	p.Glu487fs	0.0001	0	15
c.1456G>T	p.Gly486Trp	0.0001	0	15
c.1432G>A	p.Asp478Asn	0.0001	0	14
c.1417A>T	p.Lys473*	0.0001	0	14
c.1355G>A	p.Arg452His	0.0001	0	14
c.1342T>A	p.Cys448Ser	0.0001	0	14
c.1256C>T	p.Thr419Met	0.0001	0	13
c.1213A>G	p.Arg405Gly	0.01	0.006	12
c.1210T>A	p.Tyr404Asn	0.0001	0	12
c.1129C>T	p.Arg377Trp	0.0001	0	11
c.1114C>T	p.Arg372Trp	0.0001	0	11
c.1108C>T	p.Leu370Phe	0.0001	0	11
c.829A>G	p.Thr277Ala	0.0001	0	10
c.692T>C	p.Ile231Thr	0.0001	0	9
c.674C>T	p.Ala225Val	0.0001	0	9
c.647A>G	p.Gln216Arg	0.0001	0	9
c.563A>T	p.Lys188Ile	0.0002	0	8
c.562A>T	p.Lys188*	0.0002	0	8
c.430A>G	p.Ser144Gly	0.0002	0	7
c.314C>T	p.Thr105Met	0.0001	0	6
c.208A>G	p.Lys70Glu	0.0001	0	4
c.202C>G	p.Pro68Ala	0.0001	0	4
c.192C>G	p.Asp64Glu	0.0001	0	4
c.176C>G	p.Thr59Ser	0.0005	0.0003	4
c.169A>G	p.Ile57Val	0.0001	0	4
c.155A>G	p.His52Arg	0.0002	0	4
c.122G>T	p.Arg41Leu	0.0002	0	4
c.71C>G	p.Pro24Arg	0.0007	0.0003	4
c.9G>A	p.Met3Ile	0.0001	0	4

ALS-associated missense changes found within EOGT in 4493 sporadic ALS patients and 1924 controls. Mutations are listed 5' to 3'; EOGT has 15 exons and is encoded on the reverse strand of chromosome 3; exons 1 to 3 are non-coding.

new association between ALS and mutations in EOGT. Glycosyltransferases are likely to be an important therapeutic target in the effort of develop disease-modifying therapies for neurodegenerative disease.

Acknowledgements

The authors would like to thank the Project MinE GWAS Consortium. We are very grateful to those ALS patients and control subjects who donated biosamples.

Funding

We acknowledge grants from the Academy of Medical Sciences, EU Framework 7 (Euro-Motor), and the JPND/MRC SOPHIA, STRENGTH and ALS-CarE projects. T.M. is supported by the University of Sheffield Lee Newton PhD studentship. J.C.-K. holds a NIHR Clinical

Lectureship and P.J.S. is supported as an NIHR Senior Investigator. This work was also supported by the NIHR Sheffield Biomedical Research Centre for Translational Neuroscience and the Sheffield NIHR Clinical Research Facility.

Competing interests

The authors report no competing interests.

References

- Akimoto Y, Comer FI, Cole RN, Kudo A, Kawakami H, Hirano H, et al. Localization of the O-GlcNAc transferase and O-GlcNAc-modified proteins in rat cerebellar cortex. *Brain Res* 2003; 966: 194–205.
- Ariga T. Pathogenic role of ganglioside metabolism in neurodegenerative diseases. *J Neurosci Res* 2014; 92: 1227–42.

- Barrier L, Ingrand S, Damjanac M, Bilan AR, Hugon J, Page G. Genotype-related changes of ganglioside composition in brain regions of transgenic mouse models of Alzheimer's disease. *Neurobiol Aging* 2007; 28: 1863–72.
- Benatar M, Wu J, Andersen PM, Lombardi V, Malaspina A. Neurofilament light: a candidate biomarker of presymptomatic amyotrophic lateral sclerosis and phenocopy. *Ann Neurol* 2018; 84: 130–9.
- Bosio A, Binczek E, LeBeau MM, Fernald AA, Stoffel W. The human gene CGT encoding the UDP-galactose ceramide calactosyl transferase (cerebroside synthase): cloning, characterization, and assignment to human chromosome 4, band q26. *Genomics* 1996; 34: 69–75.
- Breton C, Snajdrova L, Jeanneau C, Koca J, Imberty A. Structures and mechanisms of glycosyltransferases. *Glycobiology* 2006; 16: 29r–37r.
- Chuh KN, Batt AR and Pratt MR. Chemical methods for encoding and decoding of posttranslational modifications. *Cell Chem Biol* 2016; 23: 86–107.
- Cirulli ET, Lasseigne BN, Petrovski S, Sapp PC, Dion PA, Leblond CS, et al. Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. *Science* 2015; 347: 1436–41.
- Coetzee T, Fujita N, Dupree J, Shi R, Blight A, Suzuki K, et al. Myelination in the absence of galactocerebroside and sulfatide: normal structure with abnormal function and regional instability. *Cell* 1996; 86: 209–19.
- Cooper-Knock J, Jenkins T, Shaw PJ. Clinical and molecular aspects of motor neuron disease. *Colloq Ser Genom Mol Med* 2013; 2: 1–60.
- Cooper-Knock J, Moll T, Ramesh T, Castelli L, Beer A, Robins H, et al. Mutations in the glycosyltransferase domain of GLT8D1 are associated with familial amyotrophic lateral sclerosis. *Cell Rep* 2019; 26: 2298–306.e2295.
- Desplats PA, Denny CA, Kass KE, Gilmartin T, Head SR, Sutcliffe JG, et al. Glycolipid and ganglioside metabolism imbalances in Huntington's disease. *Neurobiol Dis* 2007; 27: 265–77.
- Desport JC, Torny F, Lacoste M, Preux PM, Couratier P. Hypermetabolism in ALS: correlations with clinical and paraclinical parameters. *Neurodegener Dis* 2005; 2: 202–7.
- Dodge JC, Treleaven CM, Pacheco J, Cooper S, Bao C, Abraham M, et al. Glycosphingolipids are modulators of disease pathogenesis in amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A* 2015; 112: 8100–5.
- Dorst J, Kuhnlein P, Hendrich C, Kassubek J, Sperfeld AD and Ludolph AC. Patients with elevated triglyceride and cholesterol serum levels have a prolonged survival in amyotrophic lateral sclerosis. *J Neurol* 2011; 258: 613–7.
- Dupuis L, Corcia P, Fergani A, De Aguiar JLG, Bonnefont-Rousselot D, Bittar R, et al. Dyslipidemia is a protective factor in amyotrophic lateral sclerosis. *Neurology* 2008; 70: 1004–9.
- Frenkel-Pinter M, Shmueli MD, Raz C, Yanku M, Zilberzweig S, Gazit E, et al. Interplay between protein glycosylation pathways in Alzheimer's disease. *Sci Adv* 2017; 3: e1601576.
- Garnham R, Scott E, Livermore KE, Munkley J. ST6GAL1: a key player in cancer. *Oncol Lett* 2019; 18: 983–9.
- Gundogdu M, Llabres S, Gorelik A, Ferenbach AT, Zachariae U, van Aalten DMF. The O-GlcNAc transferase intellectual disability mutation L254F distorts the TPR helix. *Cell Chem Biol* 2018; 25: 513–8.e514.
- Gyls KH, Fein JA, Yang F, Miller CA, Cole GM. Increased cholesterol in Aβ-positive nerve terminals from Alzheimer's disease cortex. *Neurobiol Aging* 2007; 28: 8–17.
- Harschnitz O, Jongbloed BA, Franssen H, Straver DC, van der Pol WL, van den Berg LH. MMN: from immunological cross-talk to conduction block. *J Clin Immunol* 2014; 34 Suppl 1: S112–9.
- Hayashi H, Kimura N, Yamaguchi H, Hasegawa K, Yokoseki T, Shibata M, et al. A seed for Alzheimer amyloid in the brain. *J Neurosci* 2004; 24: 4894–902.
- Henriques A, Croixmarie V, Priestman DA, Rosenbohm A, Dirrig-Grosch S, D'Ambrá E, et al. Amyotrophic lateral sclerosis and denervation alter sphingolipids and up-regulate glucosylceramide synthase. *Hum Mol Genet* 2015; 24: 7390–405.
- Julien JP. Neurofilaments and motor neuron disease. *Trends Cell Biol* 1997; 7: 243–9.
- Kim SM, Kim H, Kim JE, Park KS, Sung JJ, Kim SH, et al. Amyotrophic lateral sclerosis is associated with hypolipidemia at the presymptomatic stage in mice. *PLoS One* 2011; 6: e17985.
- Kitazume S, Tachida Y, Oka R, Shirogami K, Saido TC, Hashimoto Y. Alzheimer's beta-secretase, beta-site amyloid precursor protein-cleaving enzyme, is responsible for cleavage secretion of a Golgi-resident sialyltransferase. *Proc Natl Acad Sci U S A* 2001; 98: 13554–9.
- Kracun I, Kalanj S, Talan-Hranilovic J, Cosovic C. Cortical distribution of gangliosides in Alzheimer's disease. *Neurochem Int* 1992; 20: 433–8.
- Kumar A, Singh PK, Parihar R, Dwivedi V, Lakhota SC and Ganesh S. Decreased O-linked GlcNAcylation protects from cytotoxicity mediated by Huntingtin Exon1 protein fragment. *J Biol Chem* 2014; 289: 13543–53.
- Lagerlof O, Hart GW, Haganir RL. O-GlcNAc transferase regulates excitatory synapse maturity. *Proc Natl Acad Sci U S A* 2017; 114: 1684–9.
- Lairson LL, Henrissat B, Davies GJ and Withers SG. Glycosyltransferases: structures, functions, and mechanisms. *Annu Rev Biochem* 2008; 77: 521–55.
- Lee E, Giovanello KS, Saykin AJ, Xie F, Kong D, Wang Y, et al. Single-nucleotide polymorphisms are associated with cognitive decline at Alzheimer's disease conversion within mild cognitive impairment patients. *Alzheimers Dement (Amsterdam)* 2017; 8: 86–95.
- Lin CY, Lai HL, Chen HM, Siew JJ, Hsiao CT, Chang HC, et al. Functional roles of ST8SIA3-mediated sialylation of striatal dopamine D₂ and adenosine A_{2A} receptors. *Transl Psychiatry* 2019; 9: 209.
- Liu F, Iqbal K, Grundke-Iqbal I, Hart GW, Gong CX. O-GlcNAcylation regulates phosphorylation of tau: a mechanism involved in Alzheimer's disease. *Proc Natl Acad Sci U S A* 2004; 101: 10804–9.
- Ludemann N, Clement A, Hans VH, Leschik J, Behl C, Brandt R. O-glycosylation of the tail domain of neurofilament protein M in human neurons and in spinal cord tissue of a rat model of amyotrophic lateral sclerosis (ALS). *J Biol Chem* 2005; 280: 31648–58.
- McLaughlin RL, Schijven D, van Rheenen W, van Eijk KR, O'Brien M, Kahn RS, et al. Genetic correlation between amyotrophic lateral sclerosis and schizophrenia. *Nat Commun* 2017; 8: 14774.
- Morrison BM, Lee Y, Rothstein JD. Oligodendroglia: metabolic supporters of axons. *Trends Cell Biol* 2013; 23: 644–51.
- Nakagawa K, Kitazume S, Oka R, Maruyama K, Saido TC, Sato Y, et al. Sialylation enhances the secretion of neurotoxic amyloid-beta peptides. *J Neurochem* 2006; 96: 924–33.
- Ogawa M, Sawaguchi S, Kawai T, Nadano D, Matsuda T, Yagi H, et al. Impaired O-linked N-acetylglucosaminylation in the endoplasmic reticulum by mutated epidermal growth factor (EGF) domain-specific O-linked N-acetylglucosamine transferase found in Adams-Oliver syndrome. *J Biol Chem* 2015; 290: 2137–49.
- Okuyama R, Marshall S. UDP-N-acetylglucosaminyl transferase (OGT) in brain tissue: temperature sensitivity and subcellular distribution of cytosolic and nuclear enzyme. *J Neurochem* 2003; 86: 1271–80.
- Pamphlett R, Morahan JM and Yu B. Using case-parent trios to look for rare de novo genetic variants in adult-onset neurodegenerative diseases. *J Neurosci Methods* 2011; 197: 297–301.
- Rapport MM, Donnenfeld H, Brunner W, Hungund B, Bartfeld H. Ganglioside patterns in amyotrophic lateral sclerosis brain-regions. *Ann Neurol* 1985; 18: 60–7.
- Salazar-guero EF, Routbort MJ, Martin J, Dawson G, Roos RP. Polyclonal IgM anti-Gm1 ganglioside antibody in patients with motor-neuron disease and variants. *Ann Neurol* 1990; 27: 558–63.
- Schneider JS. Altered expression of genes involved in ganglioside biosynthesis in substantia nigra neurons in Parkinson's disease. *PLoS One* 2018; 13: e0199189.

- Seyfried TN, Choi H, Chevalier A, Hogan D, Akgoc Z, Schneider JS. Sex-related abnormalities in substantia nigra lipids in Parkinson's disease. *ASN Neuro* 2018; 10: 1759091418781889.
- Stevens A, Weller M and Wietholter H. A characteristic ganglioside antibody pattern in the CSF of patients with amyotrophic-lateral-sclerosis. *J Neurol Neurosurg Psychiatry* 1993; 56: 361–4.
- Vajn K, Viljetic B, Degmecic IV, Schnaar RL, Heffer M. Differential distribution of major brain gangliosides in the adult mouse central nervous system. *PLoS One* 2013; 8: e75720.
- van der Spek RAA, van Rheenen W, Pulit SL, Kenna KP, van den Berg LH, Veldink JH. The project MinE databrowser: bringing large-scale whole-genome sequencing in ALS to researchers and the public. *Amyotroph Lateral Scler Frontotemporal Degener* 2019; 20: 432–40.
- Wani WY, Ouyang XS, Benavides GA, Redmann M, Cofield SS, Shacka JJ, et al. O-GlcNAc regulation of autophagy and alpha-synuclein homeostasis; implications for Parkinson's disease. *Mol Brain* 2017; 10: 32.
- Wu GS, Lu ZH, Kulkarni N and Ledeen RW. Deficiency of ganglioside GM1 correlates with Parkinson's disease in mice and humans. *J Neurosci Res* 2012; 90: 1997–2008.
- Yamaguchi T, Yamauchi Y, Furukawa K, Ohmi Y, Ohkawa Y, Zhang Q, et al. Expression of B4GALNT1, an essential glycosyltransferase for the synthesis of complex gangliosides, suppresses BACE1 degradation and modulates APP processing. *Sci Rep* 2016; 6: 34505.
- Yamamoto N, Fukata Y, Fukata M, Yanagisawa K. GM1-ganglioside-induced Abeta assembly on synaptic membranes of cultured neurons. *Biochim Biophys Acta* 2007; 1768: 1128–37.
- Yamamoto N, Matsubara T, Sato T, Yanagisawa K. Age-dependent high-density clustering of GM1 ganglioside at presynaptic neuritic terminals promotes amyloid beta-protein fibrillogenesis. *Biochim Biophys Acta Biomembr* 2008; 1778: 2717–26.
- Yanagisawa K, Ihara Y. GM1 ganglioside-bound amyloid beta-protein in Alzheimer's disease brain. *Neurobiol Aging* 1998; 19: S65–7.
- Yang CP, Li X, Wu Y, Shen Q, Zeng Y, Xiong Q, et al. Comprehensive integrative analyses identify GLT8D1 and CSNK2B as schizophrenia risk genes. *Nat Commun* 2018; 9: 838.
- Yang JW, Kim SM, Kim HJ, Kim JE, Park KS, Kim SH, et al. Hypolipidemia in patients with amyotrophic lateral sclerosis: a possible gender difference? *J Clin Neurol* 2013; 9: 125–9.
- Yuan AD, Rao MV, Nixon RA. Neurofilaments at a glance. *J Cell Sci* 2012; 125: 3257–63.
- Zitman FMP, Todorov B, Verschuuren JJ, Jacobs BC, Furukawa K, Furukawa K, et al. Neuromuscular synaptic transmission in aged ganglioside-deficient mice. *Neurobiol Aging* 2011; 32: 157–67.
- Zoller I, Bussov H, Gieselmann V and Eckhardt M. Oligodendrocyte-specific ceramide galactosyltransferase (CGT) expression phenotypically rescues CGT-deficient mice and demonstrates that CGT activity does not limit brain galactosylceramide level. *Glia* 2005; 52: 190–8.